MINOTECH T7 RNA Polymerase

Catalogue No **802-1, 5000 U**

Concentration **50 u/μl**

Store at -20°C.

Reagents supplied: 10x T7 Buffer (contains 7.5mM $MgCl_2$).

Source: Purified from an *E. coli* strain carrying a plasmid with the cDNA o RNA polymerase of T7 phage.

Description: MINOTECH T7 RNA polymerase is an efficient RNA polymerase able to transcribe DNA sequences under the T7 phage promoter up to (6 kbs).

Unit definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol ATP into acid-insoluble material in a total reaction volume of 50 μl in 1 hour at 37°C

Quality Control Assays:

- Functional Assay: T7 RNA polymerase has been tested on PCR products, annealed oligos and digested or supercoiled plasmid DNA containing T7 promoter Sequence: TAATACGACTCACTATA. 200 units of enzyme incubated with the various substrates obtaining transcripts ranging from 120 -6000 nucleotides. The full length expected transcripts were found to be above 95% pure based on an ethidium bromide-stained agarose gel.

- Absence of contaminants: Tested extensively for the absence of endo- and exodeoxyribonucleases.

Guaranteed stability: T7 RNA polymerase is guaranteed to maintain stability for six months from the date of shipment when stored as directed.

Recommended T7 reaction:

10x pol T7 buf.	2 μl
20mM RNTPs mix	2 μl
RNAseOut	0.5 μl (*NEB,M0314*)
Template DNA*	200 ng - 2 μg
MINOTECH T7 (50 u/μl)	2 μl
Sterile ultrapure water	Up to 20 μl

**PCR products/annealed oligos or plasmid DNA containing T7 promoter.*

Recommended T7 conditions:

Incubate at 37 ^{0}C for 2 hours.
Optionally add 0,5 -1 u DNAse I and incubate for 15 min at 37 ^{0} C to remove input DNA.
Purify RNAs by standard phenol chloroform protocol described in Maniatis et al., 1982.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual, p. 194-197. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y